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Na⁺ more strongly inhibits DNA compaction by spermidine (3+) than K⁺

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Abstract

The protective effects of alkali metal ions (Li⁺, Na⁺, K⁺, Rb⁺ and Cs⁺) against spermidine-induced DNA compaction were studied using single-molecule observations. We found that all alkali metal salts prevent DNA compaction, where Na⁺ more strongly prevented DNA compaction than other alkali metal ions. We discuss our results in terms of changes in ionic radii in relation to the net translational entropy of small ions due to ionic exchange between trivalent and monovalent cations.

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1. Introduction

The folding/unfolding transition of DNA molecules has attracted much attention in relation to various biological functions of DNA, such as replication and transcription [1-3]. It is well known that a variety of cationic chemical species with low and high molecular weights, such as polyamine spermidine, cationic proteins, and histones, play an essential role in the packing of long DNA chains into the eukaryotic nucleus [4,5]. In viruses, DNA is compacted by polyamine inside the capsid. Such DNA compaction is affected by environmental parameters such as ionic strength [6]. Na^+ and K^+ , the most abundant cations, are vital for various cellular functions [7-13]. For instance, monovalent cations are known to induce structural change of chromatin [14,15].

Although there have been many reports on the specific interaction of monocations with DNA [16-18], the effects of monocations on the compaction of giant DNA by multivalent cations have not yet been fully clarified. In this study, we investigated the spermidine-induced compaction of single DNA molecules in the presence of alkali metal salts (LiCl, NaCl, KCl, RbCl, and CsCl) by observations of single-molecule fluorescence microscopy.

2. Experimental section

2.1. Materials

Bacteriophage T4 DNA (166 kbp, 57 μm) was purchased from Nippon Gene Co., LTD (Toyama, Japan). The fluorescent dye YOYO-1 (Excitation/Emission = 491/509) was obtained from Molecular Probes Inc. (Oregon, USA). Spermidine-HCl was obtained

from Nacalai Tesque (Kyoto, Japan). The antioxidant 2-mercaptoethanol (2-ME) and other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Methods

Samples for fluorescent microscopy were illuminated with 490 nm visible irradiation, and fluorescence images of DNA molecules were observed using a Zeiss Axiovert 135 TV microscope equipped with a 100 x oil-immersed lens and recorded on a videotape through a Hamamatsu SIT TV camera. All observations were carried out at room temperature. The population of DNA molecules in the coil or compact state was determined by an analysis of at least 100 DNA molecules.

2.3. Sample solutions

For fluorescence microscopic measurements, T4 phage DNA was dissolved in 10 mM Tris-HCl buffer solution (0.1 μ M in nucleotide units) with 0.1 μ M YOYO-1 and 4 % (v/v) 2-ME at pH 7.4. The influence of YOYO is negligible at this concentration according to our earlier observations [19].

3. Results

Figure 1 shows fluorescence images of T4 DNA molecules moving freely in solution. Individual DNA molecules are observed as elongated coils in Tris-HCl buffer solution (Fig. 1a) and compacted globules in 1 mM spermidine (Fig. 1b). With an increase in the concentration of alkali metal salts, partially globular (segregated) DNA is generated, in which a compacted part and an elongated part coexist in a single molecule (Fig. 1c). As the salt concentration is further increased, all the DNA molecules are unfolded into elongated coil state (Fig. 1d).

Figure 2 exemplifies the distribution of long axis length of T4 DNA in the absence and presence (1 mM) of spermidine (3+) as deduced from single DNA observations. In agreement with the past studies, we observed the large discrete transition between coil and globule states with the increase in spermidine (+3) concentration [20].

Figure 3 summarizes the distribution of the long-axis length of DNAs in the presence of 1 mM spermidine at different concentrations of alkali metal salts, indicating that monovalent metal cations exert an inhibitory effect on spermidine-induced DNA compaction. This inhibitory effect is remarkable for ions with a smaller radius.

Figure 4 shows the ratio of DNA globules depending on the concentration of alkali metal salts. Our results show that Na^+ is twice as effective at preventing DNA compaction than K^+ at 30 mM. Li^+ has an only slightly weaker effect than Na^+ . The differences among the larger ions (Rb^+ , Cs^+ and K^+) were not as profound as that between K^+ and Na^+ . The data in Fig. 4 are summarized in Fig. 5 with regard to the dependence of the “half-unfolding transition concentration” (the salt concentration when 50 % DNA molecules exist as the fully globular state) on the ionic radius of the alkali atom, the salt of which was used to inhibit DNA compaction. In the sequence from Na^+ to Cs^+ , there is an almost linear correlation between ion size and potential for the inhibition of DNA compaction except for Li^+ . These results suggest that alkali metal cations, with the exception of Li^+ , with a smaller radius tend to more effectively inhibit the compaction of giant DNA molecules by multivalent cations.

4. Discussion

In the present study, we examined the effects of a monovalent salt on the conformational transition of giant DNA caused by a polycation. It has been shown that smaller monovalent cations more potently inhibit this compaction, with the exception of lithium ion. Various physico-chemical observations [21-24] and computer simulations [25] have shown that an increase in the ionic radius of a monocation leads to stronger binding to DNA. However, these past studies did not take into account the conformational state and conformational transitions of DNA, because most of the past studies treated short oligomeric DNA.

A recent paper discussed the efficiency of monovalent cations in DNA compaction in the crowded environment of a hydrophilic polymer, such as polyethylene glycol (PEG) [26]. In contrast to our observations, the report demonstrated that the compaction of DNA in PEG is enhanced in the presence of a monocation. To explain this seemingly contradictory effect of monocations in spermidine and PEG solutions, it should be noted that a coexisting salt has opposite effects in the two different condensing agents; multivalent cations and the polymer. It was found [26] that Na^+ more strongly promotes DNA compaction than K^+ , i.e., DNA molecules are folded into the compact state at a fix concentration of PEG with a smaller concentration of Na^+ than K^+ . Furthermore, the ability to promote the compaction by monovalent cations decreases with an increase in the ionic radius. Thus, it becomes apparent that the effect of a monovalent salt is less profound for larger ions in both cases; multication-induced compaction and polymer-induced compaction in a crowded environment, regardless of the seemingly opposite effect.

In the case of polymer-induced compaction, the main driving force of the DNA compaction transition is depletion force, which gives an entropic gain due to a so-called crowding effect. Since DNA is highly charged, to achieve compaction, it is necessary to neutralize the charge of DNA [27]. The promotion of compaction at a higher monovalent salt concentration corresponds to the fact that the entropic cost of charge neutralization is smaller at a higher salt concentration. It is reasonable to expect that the neutralized state of DNA will be more stable with smaller monovalent cations because of the stronger Coulomb attraction between monocations and negatively charged DNA. This explains the stronger promoting effect of smaller monovalent ions in a crowded environment.

On the other hand, the compaction of DNA induced by multivalent cations is accompanied by a significant degree of ion exchange between monovalent and multivalent cations in the vicinity of the negatively charged DNA polyelectrolyte [28]. The importance of ion exchange is revealed by the folding transition into a compact state at a fixed concentration of multivalent cation with the increase in temperature. This temperature effect can be explained by the gain in the translational entropy of the released monovalent cations from the vicinity of the polyelectrolyte chain at higher temperatures [29]. It may be natural to expect that Na^+ exhibits a higher binding ability than K^+ , corresponding to the experimental trend that Na^+ possesses a greater compaction inhibition potential than K^+ .

Based on the above scenario, we would like to discuss the effect of monovalent cations on DNA compaction induced by multivalent cations in a semi-quantitative manner. In some past representative reports, it has been mentioned that DNA condensation is

induced by the addition of multivalent cations with a valency of 3+ and 4+[30,31]. The mechanism of such DNA condensation has often been discussed in relation to the so-called counter ion condensation theory (CIC theory). The CIC theory [32,33] is applicable to ion distribution for a disperse state such as the coil state of DNA. However, the CIC theory becomes unsuitable for interpretation in a densely packed state in a polyelectrolyte. According to electrophoresis experiments that involve single-chain DNA observation, the negative charge along a DNA chain survives even just before the DNA transition into the compact state, whereas the DNA negative charge almost disappears in the compact state just after the transition [34]. Therefore, to understand DNA compaction, it is necessary to consider the re-distribution of ions. As for the details on physical chemistry of DNA compaction including this effect, we have discussed it in a review [35]. For simplicity, we assume that a counter-ion in the compact state is classified into two regions. Under this assumption, the free energy of a single chain can be written as

$$F = F_{\text{elastic}} + F_{\text{trans}} + F_{\text{ele-st}} \quad (1)$$

where F_{elastic} is the elastic free energy, F_{trans} is the translational entropy of small ions in the system and $F_{\text{ele-st}}$ is the electrostatic free energy. The first term can be written as

$$F_{\text{elastic}} = \frac{3}{2} kT (\alpha^2 + \alpha^{-2}) \quad (2)$$

where T is temperature, k is the Boltzmann constant and α is a swelling parameter, which is the expansion factor of the chain conformation from a Gaussian chain. The effective radius-dependence of free energy in this term should be negligible.

The second term can be written as

$$F_{\text{trans}} = kT \sum_{i=3+,1+,1-} n_i \log \frac{n_i}{c_i \nu} \quad (3)$$

where subscripts i designate 3+, 1+, and 1-, n_i is the number of condensed ions around a DNA chain, c_i is the concentration of ions in the bulk solution and ν is the volume of the condensed region. We ignore the change in c_i by considering the very low concentration of DNA in our experiments. For simplicity in this discussion, we decompose the electrostatic free energy to several contributions as

$$F_{\text{ele-st}} \sim \sum_{l,m} F_{\text{corr}}^{l,m} + F_{\text{screening}} \quad (4)$$

Subscript l, m designate DNA, 3+, 1+, 1-. $F_{\text{corr}}^{l,m}$ represents the contribution of the correlation between l, m in the condensed region. $F_{\text{screening}}$ represents the contribution of small ions in bulk to screening of the DNA charge. In these terms, the dominant contribution that depends on the monovalent cation size should be the correlation term between DNA and monovalent cations, since the excluded volume effect that is not derived from Coulomb interaction should be greater in an attractive case than in a repulsive case. The term for the correlation between DNA and monovalent cations in the coil state can be written for simplicity as

$$F_{\text{corr}}^{\text{DNA},1+}(n_{1+}) \sim \frac{Q n_{1+} e^2}{\epsilon} \log \left(\frac{R_{\text{DNA}} + r_{1+}}{R} \right) \quad (5)$$

where Q is the total charge number of a single DNA chain, R_{DNA} is the DNA radius, r_{1+} is the effective ion radius of monocation, and R is the average distance between DNA

chains. The electrostatic term contributes to the counter-ion condensation around DNA, and this correlation term should make the greatest contribution to the promotion or inhibition of monovalent cation condensation in the electrostatic term.

When the compact state is almost fully neutralized by a multivalent cation, the change in free energy of a monovalent cation that accompanies decompaction is roughly estimated as

$$\Delta F_{1+} = F_{1+}^{\text{coil}} - F_{1+}^{\text{compact}} \sim \frac{Q n_{1+}^{\text{coil}} e^2}{\epsilon} \log \left(\frac{R_{\text{DNA}} + r_{1+}}{R} \right) + k T n_{1+}^{\text{coil}} \log \frac{n_{1+}^{\text{coil}}}{c_{1+} \nu} + \Delta F_{1+}^{\text{other}} \quad (6)$$

The first two terms are increasing and decreasing functions of r_{1+} and c_{1+} , respectively.

$\Delta F_{1+}^{\text{other}}$ includes the contribution except for these terms. For simplicity, we assume that the total difference in free energy except for the above first two terms is insensitive to the size of a monovalent cation. Under this expectation, monovalent ions with a larger radius should require higher bulk concentrations to inhibit DNA compaction, corresponding to our observations as in Fig. 5. In other words, a monovalent cation with a large radius can compensate for the loss of entropy. As for the anomaly of Li^+ ion in the series of monovalent cations, we expect that its significant hydration effect owe to the very small ionic radius induces large stabilization in the bulk aqueous phase, and that such strong hydration effect causes penalty of Li^+ ion binding to DNA. As for the promotional effect of monovalent cation on the DNA compaction by PEG, similar argument is expected to hold. It may be natural to assume that the gain in electrostatic energy in the compact state by the binding with monovalent cation is larger.

Acknowledgement

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Figure captions

Fig. 1. Fluorescence images of T4 DNA molecules in elongated coil states (a) and compacted states at 1 mM spermidine (b). Coexistence of these two states with 50 mM NaCl (c) and an extended structure at a NaCl concentration of 150 mM (d)

Fig. 2. Distributions of long-axis length L of T4 DNA in the coil (control: white) and globule (with spermidine: black) states in 10 mM Tris-HCl buffer at pH 7.4.

Fig. 3. Distribution of long-axis length L of DNA in the presence of spermidine at four concentrations of salts. Histograms correspond to the DNA in globule (black), partial globule (gray), and coil (white) states.

Fig. 4. T4 DNA unfolding curves for LiCl (\circ), NaCl (\blacktriangle), KCl (\blacksquare), RbCl (\triangleright), and CsCl (\diamond) in 1 mM spermidine, shown as the dependence of the globule fraction (F_g) in the ensemble of DNA molecules on the salt concentration.

Fig. 5. Correlation between the concentration of alkali metal salts needed to achieve the half-unfolding transition, $[MCl](F_g=50\%)$, and the ionic radius r of alkali metal atoms.

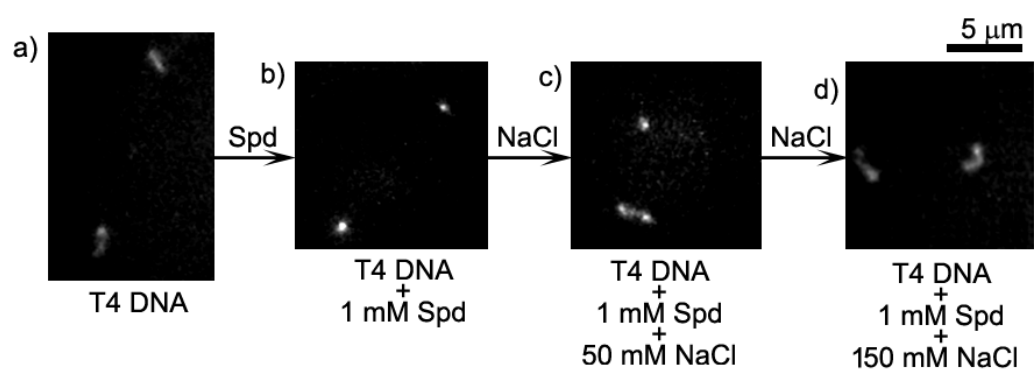


Fig. 1.

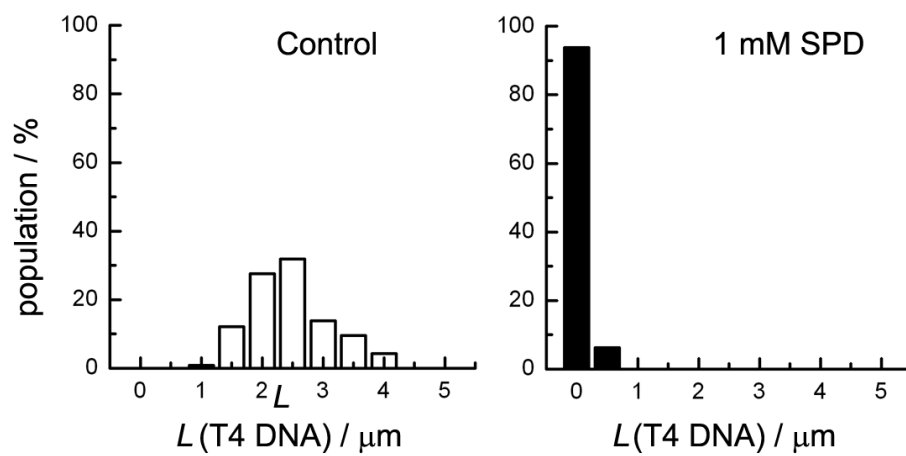


Fig. 2.

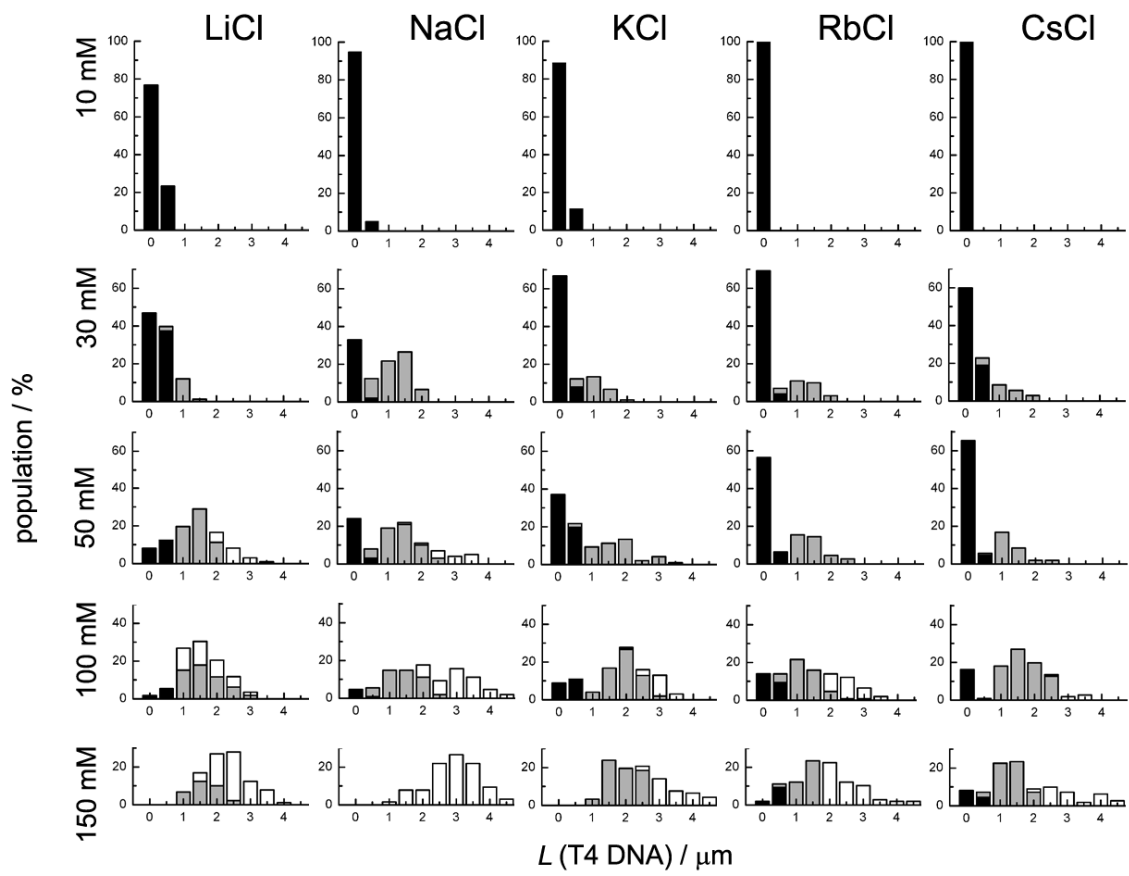


Fig. 3.

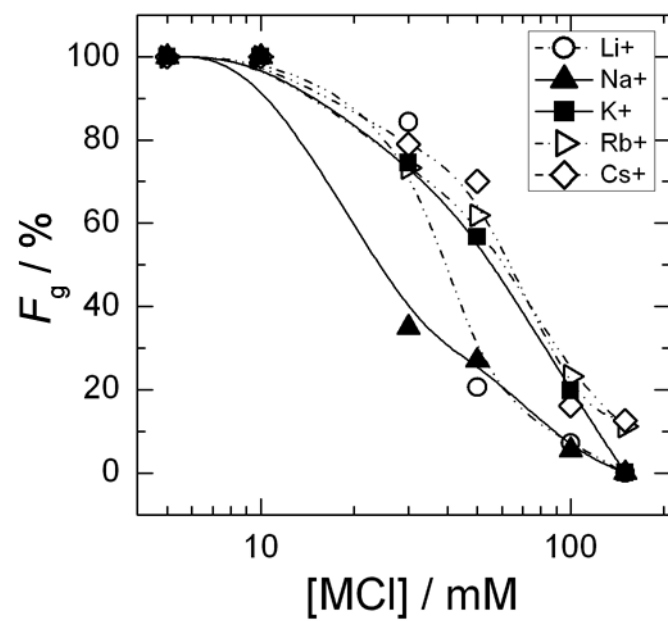


Fig. 4.

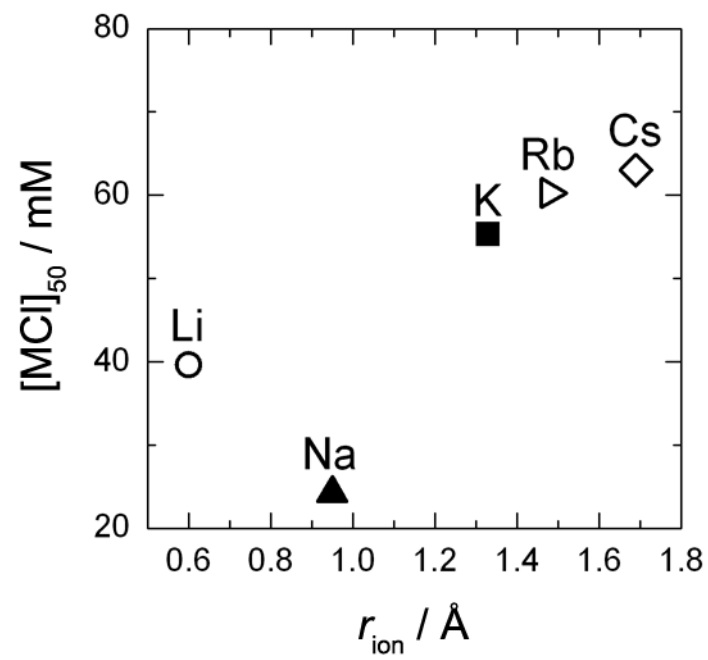


Fig. 5.

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